REMARKS

A. Summary of the Invention

In one aspect, the present invention relates to a method of determining the identity of one or more nucleotide bases at a plurality of specific positions in one or more nucleic acid molecules of interest. The method comprises the step of treating a sample comprising the nucleic acid molecules of interest if the nucleic acid molecules are double stranded so as to obtained unpaired nucleotide bases spanning the specific positions. Alternatively, a sample of the nucleic acid of interest may be used directly if the nucleic acid molecules are single stranded. The method of the invention further comprises the step of contacting the sample with a plurality of different oligonucleotide primers. Each different oligonucleotide primer hybridizes under high stringency hybridization conditions to a corresponding different stretch of nucleotide bases present in the nucleic acid molecules of interest which is immediately adjacent to the specific position of a nucleotide base to be identified with that oligonucleotide primer, so as to form a duplex such that the nucleotide base to be identified is the first unpaired base of the nucleic acid molecule of interest immediately downstream of the 3' end of the primer. Each different oligonucleotide primer comprises a corresponding different affinity moiety. The oligonucleotide primer comprising the affinity moiety is capable of hybridizing with a nucleic acid template and undergoing a nucleic acid template-dependent primer extension reaction with terminators of a terminator reagent. The affinity moiety permits affinity separation of the extended oligonucleotide primer from the terminator reagent. The method of the invention includes the further step of contacting the duplexes with a terminator reagent which includes four different terminators of a nucleic acid template-dependent primer extension reaction. The terminator reagent is free of dATP, dCTP, dGTP, and dTTP. Each terminator comprises a different detectable label corresponding to the terminator. One of the terminators is complementary to a nucleotide base to be identified by each of the oligonucleotide primers. The contacting is carried out in a primer extension reaction medium under conditions sufficient to permit a template dependent primer extension reaction, which incorporates the complimentary terminator onto the

3' end of each of the different oligonucleotide primers to thereby extend the 3' end of each of the primers by one terminator. The method of the invention further includes the step of affinity separating the respective extended oligonucleotide primers from the primer extension reaction medium by causing each of the extended oligonucleotide primers to contact an affinity group attached to a solid support. The affinity group is complementary to the affinity moiety incorporated in the oligonucleotide primer. Finally, the method of the invention comprises the step of determining the presence and identity of the nucleotide base at each of the respective specific positions in the one or more nucleic acid molecules of interest by detecting the detectable label of the terminator incorporated at the 3' end of each of the affinity separated extended oligonucleotide primers.

B. Summary of the Outstanding Office Action

Claims 64, 66, 67, and 69 through 70 inclusive were finally rejected in the Office Action of 19 May 2005 under 35 U.S.C. § 103(a) as unpatentable over European published patent application EP 0 412 883 A1 to Cohen *et al.* ("the Cohen *et al.* '883 published European application") or French patent 2,650,840 also to Cohen *et al.* ("the Cohen *et al.* '840 French patent"), each in view of international PCT published patent application WO 90/11372 to Davis *et al.* ("the Davis *et al.* '372 PCT published application").

It was noted in the Office Action that the Cohen *et al.* '883 published European application claimed priority to a French patent application 8910802, which issued as the '840 French patent. Since both the Cohen *et al.* '883 published European application and the Cohen *et al.* '840 French patent are in the French language and since an English translation of the '840 patent has been provided in the present case, only the '840 French patent as translated will be referred to specifically in the discussion which follows.

It was asserted in the outstanding Office Action that the '840 French patent disclosed a method of determining the identity of one or more nucleotide bases in a nucleic acid molecule which involved contacting a single-stranded nucleic acid sample with an oligonucleotide primer

to form a duplex between the primer and complementary target nucleic acids present in the sample, wherein the primer hybridized immediately 3' of the nucleotide to be determined. It was asserted that the method of the '840 French patent further included the step of contacting the duplexes with a solution containing four different terminators, each labeled with a different detectable moiety. The method of the '840 French patent assertedly further included the steps of extending the primer with the terminator and determining the identity of the incorporated terminator to determine the identity of the nucleotide base. It was conceded in the Office Action of 19 May 2005 that the Cohen *et al.* '840 French patent did not disclose performing the primer extension reaction using multiple primers, each comprising a different affinity moiety.

It was asserted in the Office Action of 19 May 2005 that the Davis *et al.* '372 PCT published application disclosed a method for determining the identity of one or more nucleotide bases in a nucleic acid molecule which comprised contacting a single-stranded nucleic acid molecule with an oligonucleotide primer to form a duplex between the primer and complementary target nucleic acids. It was asserted that the duplexes were contacted with a solution containing labeled dNTPs to extend the primer with the dNTPs, assertedly such that if the primer were perfectly complementary with the target nucleic acid, an extension product would be formed, but if the primer contained a mismatch at or near the 3' end of the primer, an extension product would not be formed. It was asserted in the outstanding Office Action that, in the method of the '372 PCT published application, the presence of an extension product was detected in order to determine the identity of a nucleotide base. In the Office Action, it was asserted that the '372 PCT published application disclosed that the identity of multiple nucleotides could be determined simultaneously by using a mixture of different oligonucleotides, in which each oligonucleotide comprised a unique tail. It was asserted that, following the extension reaction, the primer extension/target nucleic acid complex was denatured and the primer extension product was hybridized to a solid support having bound thereto sequences complementary to the primer tail. It was asserted in the Office Action of 19 May 2005 that the unique tail allowed for the primers to be immobilized at specific locations on the support.

It was asserted in the Office Action of 19 May 2005 that it would have been obvious to have modified the method of the Cohen *et al.* '840 French patent so as to have used multiple primers, each having a different tail, and to have separated the primer extension products from the reaction medium by contacting the extension products with a solid support having immobilized thereon nucleic acid with a sequence complementary to the tail sequence – referred to in the Office Action as a "capture probe" – assertedly in order to accomplish objectives assertedly set forth in the Davis *et al.* '372 PCT published application.

It was noted in the outstanding Office Action that a rejection of claims of the subject application as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application had been traversed in an earlier reply filed on 3 March 2005 on behalf of the applicants on the grounds that the Cohen *et al.* French patent taught away from the combination of the patent and the Davis *et al.* PCT published application of the rejection. It was noted in the 3 March 2005 reply that the Cohen *et al.* '840 French patent taught that immobilization of nucleic acids on a membrane was a disadvantage of both the method of Southern blotting and the method of United States patent No. 4,656,127 to Mundy ("the Mundy '127 patent"). It was reasoned that a person of ordinary skill in the art would have recognized that the method of the Davis *et al.* '372 PCT published application required immobilization of nucleic acid and therefore shared the disadvantage of previously known techniques requiring immobilization on a membrane which the Cohen *et al.* '840 French patent expressly taught was advantageous to avoid.

In the Office Action of 19 May 2005, with a quotation from page 3, lines 3 through 17 of the Cohen *et al.* '840 French patent, it was acknowledged that the Cohen *et al.* patent taught away from using Southern blot hybridization techniques to detect single nucleotide variations. There appears to be no corresponding acknowledgement in the Office Action that the Cohen *et al.* '840 French patent also taught away from the Mundy '127 patent, which, for embodiments characterized as preferred, disclosed immobilization of nucleic acid on a membrane using a direct spotting technique more similar to the technique disclosed in the Davis *et al.* '372 PCT

published application for immobilizing nucleic acid than the gel-to-membrane transfer technique of Southern blotting. Without referring to a distinction drawn in the Cohen *et al.* French patent between a "long probe" method and a "short probe" method, it was asserted in the Office Action that the method of Southern was substantially different from the method of the Davis *et al.* PCT published application, with the comment that the method of the Southern relied solely on the selection of suitable hybridization conditions to distinguish between nucleic acids having a single nucleotide difference. With no citation to technical literature, it was asserted that in conventional Southern blotting techniques, the sample nucleic acid was irreversibly bound to a membrane via baking or UV crosslinking. It was asserted that the method of the Davis *et al.* published application did not rely on the selection of appropriate hybridization conditions to identify the presence of a single nucleotide variation, but instead detected such a variation by a primer extension reaction. It was asserted that the method of the Davis *et al.* '372 PCT published application did not require irreversible immobilization of target nucleic acids onto a solid support, but required reversible immobilization of primer extension products onto a support via binding with an affinity reagent – which was disclosed in the application to be an oligonucleotide complementary to a tail sequence included in a primer extension product. Without mentioning how the nucleic acid capture probes were attached to the solid support, it was asserted in the 19 May 2005 Office Action that the immobilization step in the Davis *et al.* published application did not require baking or UV crosslinking. It was asserted in the Office Action that the step of hybridizing the primer extension product to an immobilized capture probe in the method of the Davis *et al.* published application differed significantly from the step of hybridizing a probe to target nucleic acid in the method of Southern, with the comment that standard hybridization conditions could be employed in the method of Davis *et al.* published application, assertedly in contrast to the method of Southern in which the detection process relied on the selection suitable hybridization conditions which would allow the probe to hybridize only if it were perfectly complementary to the target nucleic acid. While it was recognized in the Office Action of 19 May 2005 that the Cohen *et al.* '840 French patent taught away from irreversibly immobilizing a target nucleic acid and directly detecting a single nucleotide variation by hybridization of a target

nucleic acid to a probe, it was asserted that there was nothing in the Cohen *et al.* French patent which taught away from reversibly immobilizing a primer extension product as a means for distinguishing multiple primer extension products.

In the Office Action of 19 May 2005, it was asserted that the Davis *et al.* '372 PCT published application and the Cohen *et al.* '840 French patent were analogous art, with the comment that both the method of the Davis *et al.* published application and the method of the Cohen *et al.* patent assertedly relied on performing a primer extension reaction to detect a single nucleotide variation. It was asserted that the Cohen *et al.* patent and the Southern method did not rely on similar techniques to accomplish the detection of a single nucleotide variation, with the comment that the Cohen *et al.* patent disclosed detecting a single nucleotide variation using a primer extension reaction and the Southern method involved detecting a single nucleotide variation using a hybridization reaction. In the Office Action of 19 May 2005, no mention was made of the Mundy '127 patent in connection with so-called analogous art – even though the method of the Mundy '127 patent involved performing a primer extension reaction to detect a single nucleotide variation and the Mundy patent disclosed essentially the same direct spotting technique for immobilizing nucleic acid on a membrane as the Davis *et al.* published application disclosed for immobilizing oligonucleotides on a membrane for hybridizing primer extension products.

It was asserted in the 19 May 2005 Office Action that it would have been obvious to one of ordinary skill on the art at the time the invention was made to have modified the method of the Cohen *et al.* '840 French patent so as to have used multiple primers, each having an affinity moiety, and to have separated the primer extension products from the reaction medium by contacting the extension products with a solid support, in order to have accomplished the objectives assertedly set forth in the Davis *et al.* '372 PCT published application of allowing for the analysis of multiple sequences simultaneously and providing a more rapid and sensitive means for determining the identity of a nucleotide.

Claim 68 was finally rejected in the Office Action of 19 May 2005 under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '883 published European application or the Cohen *et al.* '840 French patent, each in view of the Davis *et al.* '372 PCT published application and United States patent No. 5,332,666 to Prober *et al.* ("the Prober *et al.* '666 patent"). It was conceded in the outstanding Office Action that the hypothetical combination of the Cohen *et al.* patent and the Davis *et al.* published application proposed in the Office Action did not disclose using a terminator that comprised arabinoside triphosphate. It was asserted that the Prober *et al.* '666 patent disclosed that a terminator may contain an arabinose as the sugar group. It was asserted in the 19 May 2005 Office Action that it would have been obvious to one of ordinary skill in the art to have modified the method of the Cohen *et al.* '840 French patent so as to have a terminator comprising an arabinoside triphosphate.

It was asserted in the Office Action that the comments with respect to the position taken on behalf of the applicants in the previous reply of 3 March 2005 summarized above applied equally to the position taken on behalf of the applicants in that previous reply with respect to the rejection of claim 68.

Claim 71 was finally rejected in the outstanding Office Action under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '883 published European application or the Cohen *et al.* '840 French patent, each in view of the Davis *et al.* '372 PCT published application and United States patent No. 4,962,020 to Tabor *et al.* ("the Tabor *et al.* '020 patent"). In the Office Action it was stated that the hypothetical combination of the Cohen *et al.* '840 French patent and the Davis *et al.* '372 PCT published application proposed in the Office Action did not disclose including pyrophosphatase in the primer extension medium. It was asserted that the Tabor *et al.* '020 patent disclosed including pyrophosphatase in primer extension reactions to remove pyrophosphate which builds up in such reactions. The Tabor *et al.* '020 patent assertedly disclosed that, in the presence of pyrophosphate, DNA polymerase adds pyrophosphate to the 3' terminal nucleotide, assertedly causing release of dideoxynucleoside 5'-triphosphates, removing

the block at the 3' terminus. It was asserted that in the Office Action of 19 May 2005 that it would have been obvious to one of ordinary skill in the art to have modified the method of the Cohen *et al.* '840 French patent so as to have included pyrophosphatase in the reaction medium assertedly to eliminate pyrophosphorolysis activity of DNA polymerase assertedly to reduce the probability that a labeled terminator would be removed and unlabeled dideoxynucleotides would be released into the reaction medium.

It was asserted in the Office Action that the comments with respect to the position taken on behalf of the applicants in the previous reply of 3 March 2005 summarized above applied equally to the position taken on behalf of the applicants in that previous reply with respect to the rejection of claim 71.

C. Request for Reconsideration

Reconsideration of the subject application in light of the comments below is respectfully requested.

D. The Rejections Under 35 U.S.C. § 103(a)

The attorneys for the applicants stand by the position explained in the earlier reply filed on 3 March 2005 that the Cohen *et al.* '840 French patent taught directly away from the combination, proposed in Office Action rejections under 35 U.S.C. § 103(a), of the process for identifying a single base in a nucleic acid sequence of the Cohen *et al.* patent with the method of the Davis *et al.* '372 PCT published application for testing a single sample of DNA simultaneously for multiple alleles or for testing simultaneously at multiple loci for a single allele or multiple alleles.

In this regard, we note at the outset that the Cohen *et al.* '840 French patent expressly distinguished the method of the patent from three previously known techniques for identifying a mutation in nucleic acid involving a single nucleotide position: (1) a long-probe Southern blot technique, (2) a short-probe technique which it is submitted that persons of ordinary skill in the

art would have recognized to be a type of Southern-blot technique, as discussed below, and (3) the method of the Mundy '127 patent. The three known techniques distinguished from the method of the Cohen *et al.* patent in the patent specification differed fundamentally one from another in the manner in which the single nucleotide mutation was identified using labeled oligonucleotide probes, but which had in common, in embodiments to which relevant distinguishing comments in the Cohen *et al.* patent applied, steps involving detection of the labeled probes hybridized along at least a portion of their length to nucleic acid immobilized on a membrane. However, the remarks in the outstanding Office Action responding to the position that the Cohen *et al.* '840 French patent taught against the proposed combination of the process of the Cohen *et al.* patent with the method of the Davis *et al.* '372 PCT published application focused on only one of the three previously known techniques distinguished from the method of the Cohen *et al.* patent in the patent specification – specifically, the technique referred to in the Cohen *et al.* patent as the short-probe Southern blot technique.

Additionally, as discussed in more detail in the following subsection, the remarks in the outstanding Office Action emphasized differences between the manner in which the single nucleotide mutation was identified in the short-probe Southern blot technique – an allele-specific hybridization technique involving a labeled probe where a match or mismatch occurred in the vicinity of the center of the probe – and the manner in which such a mutation was identified in the Davis *et al.* published application – an allele-specific primer-extension reaction method using labeled chain extenders where a match or mismatch occurred at the 3' end of a primer/probe – but did not discuss a feature that the short-probe Southern blot technique and the method of the Davis *et al.* published application had in common; namely, the feature the feature of ultimately detecting the presence or absence of a labeled probe hybridized to nucleic acid immobilized on a membrane. The Cohen *et al.* '840 French patent distinguished the short-probe Southern blot technique from the method of the patent not only on the basis of the difficult hybridization conditions required to achieve allele-specific hybridization in the short-probe Southern blot technique, but also on the basis of a requirement – specifically characterized in the Cohen *et al.* patent as a disadvantage – to immobilize nucleic acid on a membrane in Southern blot techniques

generally, including the short-probe Southern blot technique. As noted above, the requirement to immobilize nucleic acid on a membrane, was a distinguishing feature shared in common by the three previously known techniques distinguished from the method of the Cohen *et al.* patent in the patent specification. In teaching that it was a disadvantage to immobilize nucleic acid on a membrane in the short-probe Southern blot technique and in two other, different previously known techniques, it is submitted that the Cohen *et al.* '840 French patent taught directly away from the combination of the process of the Cohen *et al.* patent with the method of the Davis *et al.* '372 PCT published application proposed in Office Action of 19 May 2005, since, as discussed below, the method of the Davis *et al.* published application involved immobilizing nucleic acid on a membrane.

D.1 The Cohen *et al.* '840 French Patent in View of the Davis *et al.* '372 PCT Published Application

As disclosed at page 4, line 29 through page 5, line 19 of the Cohen *et al.* '840 French patent, the process of the patent for detecting a specific nucleotide base present on a nucleic acid sequence involved hybridizing the sequence in which the base to be identified was located with a "trigger" nucleotide which hybridized with its 3' end adjacent to the specific nucleotide base to be detected. Synthesis of the complementary strand of the resulting hybrid was initiated in the presence of a polymerase without 3'-to-5' exonuclease action and at least one modified nucleotide base capable of being incorporated into the extension product of the trigger nucleotide and of blocking further elongation of the extension product. The process of the Cohen *et al.* '840 French patent further involved detecting the incorporated blocking nucleotide base to identify the specific complementary nucleotide base located in the target nucleic acid sequence. According to page 5, lines 23 through 31 of the Cohen *et al.* patent, the blocking nucleotide bases could be dideoxynucleotides marked with radioactive substances, enzymes, fluorescent or chemoluminescent chromophoric chemical products, or antibodies.

At page 6, lines 29 through 33 of the Cohen *et al.* '840 French patent, it was disclosed that a purported advantage of the process of the patent was that the process did not require

immobilization of the nucleic acid on a membrane. As may be seen, for example, at page 1, lines 5 through 13, and page 2, lines 8 through 18 of the Cohen *et al.* patent, in the context of the patent, the term "nucleic acid" applied generally to each strand of hybridized DNA or RNA, including probes 150 nucleotides long and shorter probes. Moreover, as noted in the preceding section, the necessity to immobilize nucleic acid on a membrane was specifically pointed out in the Cohen *et al.* '840 French patent to be a disadvantage shared in common by the previously-known Southern blot technique generally – described in the Cohen *et al.* patent as including two substantially different variants: a long-probe variant and a short-probe variant – and the substantially different method of the Mundy '127 patent. See page 3, lines 10 through 17 and page 4, lines 14 through 17 of the Cohen *et al.* patent. It is submitted therefore that the Cohen *et al.* '840 French patent would have directly led persons skilled in the art away from any technique which shared the requirement of immobilization of nucleic acid on a membrane.

Persons of ordinary skill in the art would have recognized, it is submitted, that the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application was just such a technique involving immobilization of nucleic acid on a membrane from which the Cohen *et al.* patent taught away. The Davis *et al.* published application disclosed a technique for determining the existence or nonexistence of a test nucleotide on a strand of DNA which employed a polymerization agent capable of synthesizing an extension product if there were a match between the test nucleotide on the DNA strand and a nucleotide opposite on an extension primer, but not if there were a mismatch. According to page 5, line 19 through page 6, line 22 of the Davis *et al.* '372 PCT published application, a single sample of DNA could be tested simultaneously for multiple alleles at a single locus or for a single allele or multiple alleles at multiple loci by treating the DNA with a plurality of different oligonucleotide primers, each primer being complementary to a different allele and each having a unique oligonucleotide "tail." The primers and the DNA were then subjected to conditions that would have allowed the primers and DNA to pair and labeled extension products to form if there were a match between a test nucleotide and the opposite nucleotide on the primer, but not if there were a mismatch. It was disclosed at page 6, lines 7 through 22 and page 21, lines 8 through 12 of the Davis *et al.*

published application that the presence or absence of a particular extension product could be determined by applying the putative extension products to a substrate such as filter paper, nylon, or nitrocellulose "spotted" at distinct locations with unique oligonucleotides complementary to each of the unique oligonucleotide tails. As discussed in more detail below, the Davis *et al.* '372 PCT published application at page 8, lines 1 through 5, for example, that such spotting of oligonucleotides complementary to each of the unique oligonucleotide tails on the substrate preferably resulted in the oligonucleotides "being firmly bound to the substrate but accessible for hybridization with complementary sequences." According to the Davis *et al.* published application, if a particular extension product existed, it would have attached to the substrate at only one location by way of hybridization of the unique tail to the complementary oligonucleotide found only at that location on the substrate. The Davis *et al.* '372 PCT published application disclosed that by detecting the presence of a labeled extension product hybridized to a complementary oligonucleotide bound to the substrate at a specific location, the presence or absence of a specific allele in the test DNA could be determined.

In the Office Action of 19 May 2005, it was noted that, in conventional Southern blot techniques, sample nucleic acid was bound irreversibly to a membrane by baking or by UV crosslinking, in asserted contrast to the method of the Davis *et al.* '372 PCT published application, which involved reversibly immobilizing a primer extension product onto a solid substrate by a hybridization reaction between the primer's unique tail and an oligonucleotide complementary to the tail bound to the substrate. It was asserted in the Office Action that, while the Cohen *et al.* '840 French patent taught away from irreversibly immobilizing a target nucleic acid, there was assertedly nothing in the Cohen *et al.* patent which taught away from reversibly immobilizing a primer extension product as a means for distinguishing between multiple primer extension products.

However, the Cohen *et al.* '840 French patent drew no distinction between reversibly immobilizing nucleic acid on a membrane and irreversibly immobilizing nucleic acid on a membrane, but declared without qualification on page 6, lines 29 through 33 of the specification

that an advantage of the process of the patent was that the process did not require immobilization of the nucleic acid on a membrane. Significantly in this regard, each of the three previously known techniques for identifying a single nucleotide mutation distinguished in the Cohen *et al.* patent from the process of the patent in terms of having the disadvantage of requiring nucleic acid immobilized on a membrane would, it is submitted, have been expected by persons of ordinary skill in the art generally to have involved detecting a hybrid nucleic-acid complex immobilized on a membrane, in which a first component of the hybrid complex was a nucleic acid effectively irreversibly bound to the membrane and a second component of the hybrid complex was a labeled oligonucleotide of some sort reversibly bound to the first component by hybridization. Moreover, as discussed below, persons of ordinary skill in the art would have recognized, it is submitted, that the method of the Davis *et al.* '372 PCT published application likewise involved detecting a hybrid nucleic-acid complex immobilized on a membrane in which a first component of the hybrid complex was a nucleic acid effectively irreversibly bound to the membrane and a second component of the hybrid complex was a labeled oligonucleotide reversibly bound to the first component by hybridization and therefore such persons would have appreciated that the method of the Davis *et al.* published application necessarily entailed a feature which the Cohen *et al.* '840 French patent characterized specifically as a disadvantage not shared by the process disclosed in the Cohen *et al.* patent. Persons of ordinary skill in the art would thus have deemed it in no way obvious to combine the process of the Cohen *et al.* patent with the method of the Davis *et al.* '372 PCT published application as proposed in the Office Action of 19 May 2005.

Turning first to the technique described generally in the Cohen *et al.* '840 French patent in terms of a long-probe Southern blot technique at page 2, lines 19 through 34 and page 3, lines 10 through 17 of the patent, it is submitted that persons of ordinary skill in the art would have understood the technique to be a conventional restriction-site analysis method of which the method described in E. M. Southern, *Journal of Molecular Biology*, volume 98, pages 503 through 517 (1975) ("the Southern publication") was illustrative. A copy of the Southern publication is being submitted with the present reply. Reference to pages 504 through 508 of the

Southern publication, and to page 506, lines 7 and 8 in particular, will show that the technique involved blot transfer of denatured DNA fragments from an electrophoresis gel to a cellulose nitrate strip, after which transfer the cellulose nitrate strip was baked in a vacuum oven for two hours at 80° C. The strip bearing the denatured DNA fragments was then treated with a solution of radioactive RNA to permit the RNA to hybridize to any complementary DNA. The strip was then washed, dried, and laid on X-ray film to detect the location of any hybrid RNA/DNA complex on the strip. Any radioactive spots on the cellulose nitrate strip detected by the X-ray film would have corresponded to a hybrid nucleic-acid complex immobilized on the strip, including, as one component, denatured DNA bound to the strip by the vacuum baking step and, as the other component, radioactively labeled RNA hybridized to the bound denatured DNA. It is submitted that a person of ordinary skill in the art would have understood the long-probe Southern blot technique described generally in the Cohen *et al.* '840 French patent likewise to have involved detecting a hybrid nucleic-acid complex immobilized on a membrane, in which a first component of the hybrid complex would have been a nucleic acid effectively irreversibly bound to the membrane by vacuum baking or a process of similar effect and a second component of the hybrid complex would have been a radioactively labeled oligonucleotide reversibly bound to the first component by hybridization.

Turning next to a technique described generally passim from page 2, line 19 to page 3, line 17 of the Cohen *et al.* '840 French patent which involved use of a short probe in contrast to the longer probe of the long-probe Southern blot technique discussed in the preceding paragraph, it is submitted that persons of ordinary skill in the art would have understood from the description as a whole that the short-probe technique as described was a variant of the long-probe Southern blot technique discussed above – which for convenience is being referred to as a short-probe Southern blot technique in the present reply. Specifically, it is submitted that persons of ordinary skill in the art would have understood the described short-probe Southern blot technique to be an allele-specific hybridization method, of which one of two alternative allele-specific-hybridization methods described in B. J. Conner *et al.*, *Proceedings of the National Academy of Sciences USA*, vol. 80, pages 278 through 283 (January 1983) ("the Conner *et al.* publication")

would have been illustrative. A copy of the Conner *et al.* publication is being submitted with the present reply. (The Conner *et al.* publication disclosed an alternative allele-specific hybridization procedure on page 279, right-hand-side column, lines 1 through 8, which evidently did not involve immobilization of nucleic acid on a membrane and which therefore appears to be irrelevant to the present discussion.)

According to the abstract on page 278 of the Conner *et al.* publication, two oligonucleotides nineteen bases long, one complementary to the normal human β-globin gene and the other complementary to the sickle cell β-globin gene, could be radioactively labeled and used as probes in DNA hybridization under particular hybridization conditions. Specifically, hybridization conditions reportedly could be found such that the probes could be used to distinguish the normal gene from the sickle-cell gene on the basis of to which gene the respective probes hybridized. One method of detecting whether such allele-specific hybridization did or did not occur disclosed in the Conner *et al.* publication involved digesting a sample of DNA with a particular restriction enzyme, separating the restriction fragments on an electrophoresis gel, denaturing the DNA fragments on the gel, and transferring the denatured fragments to nitrocellulose paper "by the standard Southern procedure." See page 280, left-hand-side column, lines 2 through 7 of the Conner *et al.* publication. The reference to the standard Southern procedure quoted above was accompanied by a citation to the Southern publication discussed in the preceding paragraph. As noted in the preceding paragraph, the technique of blot transfer of denatured DNA fragments from an electrophoresis gel to a strip of nitrocellulose disclosed in the Southern publication involved baking the strip to which the fragments had been transferred in a vacuum oven.

According to page 279, left-hand-side column, line 33 through page 279, right-hand-side column, line 1 and page 279, right-hand-side column, lines 8 through 14, of the Conner *et al.* publication, hybridization of the denatured DNA fragments with the radioactively labeled oligonucleotide probes could be carried out directly on the nitrocellulose paper to which the fragments had been transferred according to the procedure of the Southern publication. Whether

or not hybridization between a labeled probe and a DNA fragment on the nitrocellulose paper occurred could be detected by autoradiography after washing the paper under particular specified conditions. Any radioactive spots on the nitrocellulose paper detected by autoradiography would have corresponded to a hybrid nucleic-acid complex immobilized on the paper, including, as one component, denatured DNA bound to the paper by the vacuum baking step called for in the Southern paper referenced in the Conner *et al.* publication and, as the other component, radioactively labeled oligonucleotide probe material hybridized to the bound denatured DNA. It is submitted that a person of ordinary skill in the art would have understood the short-probe Southern blot technique described generally in the Cohen *et al.* '840 French patent likewise to have involved detecting a hybrid nucleic-acid complex immobilized on a membrane, in which a first component of the hybrid complex would have been a nucleic acid effectively irreversibly bound to the membrane by vacuum baking or a process of similar effect and a second component of the hybrid complex would have been a radioactively labeled oligonucleotide reversibly bound to the first component by hybridization.

In the Mundy '127 patent, the method of the patent for detecting a mutation of a specific nucleotide base in a target nucleic acid chain was distinguished both from the long-probe Southern blot technique discussed above – see column 1, lines 15 through 35 of the Mundy patent – and from the short-probe Southern blot technique of the Conner *et al.* publication – see column 1, lines 36 through 50 of the Mundy patent. However, in embodiments of the method of the Mundy '127 patent characterized in the patent as preferred, the identity of the mutation would have been determined by detecting the presence or absence of a labeled probe reversibly hybridized to the target nucleic acid irreversibly bound to a nitrocellulose filter. For example, it was disclosed at column 5, lines 6 through 11 of the Mundy patent that single-stranded target chains were preferably immobilized on nitrocellulose. According to the patent, such immobilization could have been

> effected by spotting purified DNA onto nitrocellulose filters and baking at 80° C. to fix the single-stranded target, or possibly by direct processing of cells on nitrocellulose filters.

According to the abstract of the Mundy '127 patent, the method of the patent would have included the step of hybridizing a labeled probe to the target nucleic acid chain to form a hybrid in which one end of the probe would have been positioned adjacent to the specific base to be identified. Regarding the labeled probe, it was disclosed at column 5, lines 36 through 38 of the Mundy patent that the probe could have two sequences, one to hybridize to the target chain and one to carry the label. The abstract of the Mundy '127 patent disclosed that, after the hybrid complex of labeled probe and target nucleic acid chain would have been formed, a digestion-resistant nucleotide derivative such as a thionucleotide would have been added under conditions to cause it to join the end of the probe if it were complementary to the specific base. The resulting hybrid complex would have then been treated with an exonuclease enzyme under conditions such that, if present on the end of the probe, the digestion-resistant nucleotide derivative would have protected the probe from digestion. As disclosed in the abstract of the Mundy patent and at column 2, lines 21 through 23 of the patent, observation of the presence or absence of the probe label attached to the target nucleic-acid chain after the digestion step would have detected the mutation of specific base in the target chain. In embodiments of the method of the Mundy '127 patent characterized as preferred in which the target nucleic-acid chain would have been immobilized on a nitrocellulose filter by spotting and baking, the identity of the mutation would therefore have been determined by observing the presence or absence of a labeled probe reversibly hybridized to the target nucleic acid, which in turn would have been irreversibly bound to the nitrocellulose filter. It is submitted that a person of ordinary skill in the art would have understood the method of the Mundy '127 patent in embodiments characterized in the patent as preferred, like the long-probe Southern blot technique and the short-probe Southern blot technique in spite of differences noted in the Mundy patent itself, to have involved detecting a hybrid nucleic-acid complex immobilized on a membrane, in which a first component of the hybrid complex would have been a nucleic acid effectively irreversibly bound to the membrane by baking or a process of similar effect and a second component of the hybrid complex would have been a labeled oligonucleotide reversibly bound to the first component by hybridization.

It is demonstrated below that the method of the Davis *et al.* '372 PCT published application, which was proposed to be combined with the process of the Cohen *et al.* '840 French patent in the outstanding Office Action, also involved detecting a hybrid nucleic-acid complex immobilized on a membrane, in which a first component of the hybrid complex would have been a nucleic acid effectively irreversibly bound to the membrane by baking or a process of similar effect and a second component of the hybrid complex would have been a labeled oligonucleotide reversibly bound to the first component by hybridization. As pointed out above, the Davis *et al.* published application disclosed a technique for determining the existence or nonexistence of a test nucleotide on a strand of DNA which employed a polymerization agent capable of synthesizing an extension product if there were a match between the test nucleotide on the DNA strand and a nucleotide opposite on an extension primer, but not if there were a mismatch. A single sample of DNA could be tested simultaneously for multiple alleles at a single locus or for a single allele or multiple alleles at multiple loci according to the Davis *et al.* '372 PCT published application by treating the DNA with a plurality of different oligonucleotide primers, each primer being complementary to a different allele and each having a unique oligonucleotide tail. The primers and the DNA would then have been subjected to conditions that would have allowed the primers and DNA to pair and labeled extension products to form if there were a match between a test nucleotide and the opposite nucleotide on the primer, but not if there were a mismatch.

It was disclosed at page 6, lines 7 through 22 and page 21, lines 8 through 12 of the Davis *et al.* published application that the presence or absence of a particular extension product could be determined by applying the putative extension products to a substrate such as filter paper, nylon, or nitrocellulose "spotted" at distinct locations with unique oligonucleotides complementary to each of the unique oligonucleotide tails. In Example 4 of the Davis *et al.* '372 PCT published application, a procedure for spotting nylon with oligonucleotides complementary to each of the unique oligonucleotide tails was identified. Specifically, at page 51, lines 17 through 21 of the Davis *et al.* published application, it was disclosed that two poly-complements to two oligonucleotide tails could be bound to discrete locations on a nylon

membrane identified by the brand name "ZetaProbe" using conditions recommended by the manufacturer BioRad. Submitted with the present reply is a copy of an instruction manual for "Zeta-Probe® Blotting Membranes" published by Bio-Rad Laboratories of Hercules, California. The publication date of the instruction manual is not known. It nonetheless seems of interest to note that Section 2.5 on pages 8 and 9 of the manual describes procedures recommended for DNA dot blotting using Zeta-Probe nylon membranes. Step 7 of the recommended procedure calls UV-crosslinking the DNA to the membrane or vacuum drying the membrane bearing the DNA for 30 minutes at 80° C. Persons of ordinary skill in the art, it is submitted, would have understood such conditions to have led to an essentially irreversible binding of the DNA to the membrane. Whatever the status of the Zeta-Probe manual with respect to availability as of the publication date of the Davis *et al.* '372 PCT published application, the Davis *et al.* published application disclosed at page 8, lines 1 through 5 that the oligonucleotides complementary to each of the unique oligonucleotide tails were preferably "firmly bound" to the substrate and disclosed at page 22, lines 14 through 19 that such oligonucleotides were preferably covalently linked to the substrate.

In the method of the Davis *et al.* '372 PCT published application, to such oligonucleotides firmly bound, covalently linked to a substrate were hybridized the respective complementary oligonucleotide tails of oligonucleotide primers, labeled or not depending on whether there was a match or a mismatch between the 3'end of the corresponding primer at the site of a test nucleotide on a strand of DNA. The method of the Davis *et al.* published application thus involved detecting a hybrid nucleic-acid complex immobilized on a membrane, in which a first component of the hybrid complex – a unique oligonucleotide complementary to a unique oligonucleotide tail of one of the primers – would have been a nucleic acid effectively irreversibly bound to the membrane by baking or a process of similar effect and a second component of the hybrid complex – a labeled or unlabeled unique oligonucleotide primer with its unique oligonucleotide tail – would have been a labeled oligonucleotide reversibly bound to the first component by hybridization.

For the reasons set forth above, it is submitted that, assuming for the sake of argument only that the hypothetical combination of the single-base-identification process of the Cohen *et al.* '840 French patent with the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application proposed in the outstanding Office Action would even have occurred to a person of ordinary skill on the art as of the effective date of the subject application, such a person would have recognized that the method of the Davis *et al.* '372 PCT published application involved immobilizing nucleic acid on a substrate and that the method would therefore have effectively shared the disadvantage of three previously known methods requiring immobilizing nucleic acid on a membrane specifically pointed out in the Cohen *et al.* patent It is submitted therefore that a person of ordinary skill in the art would not have attempted to combine the single-base-identification process of the Cohen *et al.* '840 French patent with the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application in view of the teachings in the Cohen *et al.* patent directly away from such the hypothetical combination.

For the reasons set forth above, it is submitted that the final rejection in the Office Action of 19 May 2005 of claims 64, 66, 67, and 60 through 70 inclusive of the subject application as amended under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application was without justification and should be withdrawn.

D.2 The Cohen *et al.* '840 French Patent in View of the Davis *et al.* '372 PCT Published Application and the Prober *et al.* '666 Patent

The Prober '666 patent in no way overcomes the teachings of the Cohen *et al.* '840 French patent against the hypothetical combination of the single-base-identification process of the '840 French patent with the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application proposed in the outstanding Office Action discussed in the preceding subsection and consequently the reasoning of the preceding subsection applies equally with respect to the rejection of claim 68 in the Office Action of 19 May 2005 under

35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application and the Prober *et al.* '666 patent. It is submitted that the final rejection in the Office Action of 19 May 2005 of claim 68 of the subject application as amended under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application and the Prober *et al.* '666 patent was unjustified and should be withdrawn.

D.3 The Cohen *et al.* '840 French Patent in View of the Davis *et al.* '372 PCT Published Application and the Tabor *et al.* '020 Patent

As in the case of the Prober *et al.* '666 patent discussed in the preceding subsection, the Tabor *et al.* '020 patent in no way overcomes the teachings of the Cohen *et al.* '840 French patent against the hypothetical combination of the single-base-identification process of the '840 French patent with the multiple-allele/multiple-loci method of the Davis *et al.* '372 PCT published application proposed in the Office Action of 19 May 2005 discussed above and consequently the reasoning of the preceding subsection applies equally with respect to the rejection of claim 71 in the outstanding Office Action under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application and the Tabor *et al.* '020 patent. It is submitted that the final rejection in the Office Action of 19 May 2005 of claim 71 of the subject application as amended under 35 U.S.C. § 103(a) as unpatentable over the Cohen *et al.* '840 French patent in view of the Davis *et al.* '372 PCT published application and the Tabor *et al.* '020 patent was unwarranted and should be withdrawn.

E. Conclusion

For the reasons set forth above, it is submitted that each of the claims of the subject application is allowable over the art of record considered alone or in any combination. Withdrawal of the final rejections of the claims and allowance of the application is therefore earnestly solicited.

Respectfully submitted,

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